REMARKS/ARGUMENTS

Applicants appreciate the Examiner's time granting an interview on March 7, 2007. Applicants and Examiner discussed "flux" terminology, revising the preamble of the claims to clarify that A-coA flux is intended, and the scope of enablement.

Claims 27-33 are amended to recite "increasing Coenzyme A (CoA) flux" as appropriate and as discussed in ¶ 11 of the specification. Claim 31 was changed to recite "increases conversion of an alcohol to an ester," as suggested in ¶ 27 and 29. Finally, "isoamyl alchol" was added to claim 31 and "alcohol" changed to "acetate" as suggested by the Examiner Minor corrections to correct a lack of antecedence were also made to claims 29 and 33. Applicants have replaced "panthenoic" with "pantothenic" although the terms are equivalent, the specification uses "pantothenic" throughout.

Figure 6 & Table 2

The Examiner has objected to Fig.6 and Table 2 as confusing because the cells only express PANK, yet produce isoamyl acetate (FIG 6C). However, the plasmid pKmAT contains an acetyl transferase, thus explaining the production of isoamyl acetate in this cell line. *See e.g.,* Vidali, *et al., Applicability of CoA/acetyl-CoA manipulation system to enhance isoamyl acetate production in Escherichia coli*, Met. Eng. 6:294-9, at Table 2 (2004) (showing that pKmAT contains ATF2).

Indefiniteness Rejections

Claims 27-33 were rejected under 35 U.S.C. 112 as indefinite, particularly with respect to the preamble. Applicants discussed the claim preamble with the Examiner and agreed that amending the preamble to clarify that "increasing Coenzyme A (CoA) flux" as intended would address any possible confusion. Applicants assert that this is **not** a change in substance, only a clarification of language to use mutually agreeable terms, and the specification discusses improving CoA flux throughout.

Further, the Applicants explained that the demonstration of increased isoamyl acetate was exemplary only. In fact, this is only one of many molecules that can be converted to an ester

using the substrate non-specific ATF enzyme. Thus, there is no need to include isoamyl alcohol in the medium unless one actually desires to produce isoamyl acetate. However, the Examiner is correct that claim 31 should include isoamyl alcohol and isoamyl acetate. Applicants thank the Examiner for noting this and have made the requisite amendments.

Finally, Applicants clarify that the invention is a complex combination of cofactor manipulation and carbon flux adjustments, and that increased CoA **flux** is achieved, not increased CoA **pools**, as suggested by the Examiner at page 3 of the Office Action. Applicants trust that these amendments and clarifications have addressed each of the Examiner's indefiniteness concerns.

Written Description Rejections

The Examiner has rejected claims 27- 33 under 35 U.S.C. 112 as containing new matter because the specification allegedly does not describe the "increased production of CoA." Applicants assert that increased "flux" is produced, not steady state pools and the amended claims clarify this point. Further, increased flux **is** demonstrated by increased isoamyl acetate, as shown in Fig. 10.

Examiner has also rejected the claims as not enabled for "any" bacteria, but only enabled for *E. coli.* Please see the Declaration of George N. Bennett demonstrating the presence of conserved metabolic pathways in all bacterial species for the synthesis of coenzyme A from pantothenate and glycolytic pathways including the enzymes required to create and utilize pyruvate and acetyl-CoA. Thus the Dr. Bennett has confirmed that the claimed methods will function in **all** bacteria.

The Examiner may also reject the claims as not enabled for "any generic gene" (Office Action p. 6, also 9), but it is not clear to Applicants if this is intended to be an independent basis for rejection or is merely part of the argument supporting the rejection of "any" bacterial cell. Regardless, Applicants do **not** claim the use of generic genes, but specifically named genes that are well known in the art and encode proteins having the requisite activities (pantothenate kinase, pyruvate dehydrogenase and alcohol acetyl transferase).

There are hundreds of pantothenate kinase (PANK) genes available, and **each** PANK gene encodes a protein that **phosphorylates pantothenate**. The same is true for pyruvate dehydrogenase (PDH) and alcohol acetyl transferase (ATF). The Declaration of George Bennett provides **documentary evidence** of the functional relationship between gene name and enzymatic activity. Applicants see no reason to believe that one or more of these will fail to function in a bacteria. Efficiency of expression may vary, but it is well known how to improve expression through use of the codon preferences (see NCBI for details www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi). Further, Examiner has provided **no documentary evidence** supporting the assertion of lack of enablement, and to the extent that Examiner is relying on personal knowledge or common knowledge in the art to support **this or any other** rejection, Examiner is respectfully requested to fully articulate the rationale in proper evidentiary form according to MPEP 2144.03,[1] so that Applicants may properly rebut same.

Claims 28, 32, and 33 are rejected because allegedly Δ*ackA* or Δ*pta* is new matter, and that only the combined deletion is described. An enablement rejection on the same basis is also made, noting that Applicants "do not teach the structure of ackA and pta genes and mutation(s) that reduce the activity."

However, the specification at ¶ 28 clearly addresses the two genes in the operon separately ("A-CoA may be converted to acetyl phosphate by phosphotransacetylase (PTA), which in turn may be converted to acetate using acetate kinase (ACK)"). It is equally clear, that if **either** gene is deleted or otherwise inactivated, the reaction cannot proceed to completion. Thus, the recitation is not new matter. Further, Applicants are not required to detail what is commonly known in the art. The *ackA* and *pta* genes and their combined operon are known (see e.g., D17576 in GenBank), and it is known how to insert stop codons, delete portions of genes, and the like. This is simple genetic engineering and general methods of making chromosomal changes in bacteria have been available since the 80's![2] Thus, Applicants strongly dispute that

---

[1] MPEP 2144.03 ("If the examiner is relying on personal knowledge to support the finding of what is known in the art, the **examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support the finding**. See 37 CFR 1.104(d)(2).") (emphasis added).

[2] Gutterson NI, Koshland DE Jr., *Replacement and amplification of bacterial genes with sequences altered in vitro*, Proc Natl Acad Sci USA. 80(16):4894-8 (1983); Hamilton, *et al., New Method for generating Deletions and Gene Replacements in E. coli,* J. Bacteriol. 171:4617-22 (1989).

reduction of either *ackA* or *pta* is not enabled and dispute that experimentation is undue, and note that the Examiner has provided **no documentary evidence** for same. Applicants request that Examiner put such material in proper evidentiary form for rebuttal.

Claims 31-33 are rejected as containing new matter because "production may not be increased in results [sic] of transformation, it may only start after transformation." Applicants request clarification of this rejection, because the claim requires culture of the cell having the recited genes, not a wild type cell. The claim goes on to recite that "said combination of recombinant genes is expressed" making it clear that the cell is in fact **already** transformed. Applicants have however, amended the claim to include the substrate for the reaction.

Claim 31 is rejected for having new matter because correlation between increased "acetyl-CoA production" and isoamyl acetate is not shown. The amendments are believed to clarify that increased coA "flux" does result in increased isoamyl acetate and the requisite substrate is now recited. Thus, the rejection is believed to have been addressed.

Claims 32-33 Rejected as Obvious

Claims 32 and 33 are rejected as obvious over San in view of Vallari, Voet and Yang. However, Applicants note that no art rejection is made against claim 27, and that claim 32 has all of the elements of claim 27, plus additional elements. Thus, if claim 27 is free of the art, so should claim 33 be free of art. However, in the event that the failure to reject claim 27 was inadvertent, Applicants will address the substance of the rejection as well.

The Cited Art Does Not Teach Bacteria Having Added PANK or PDH

The claimed invention relates to a bacteria having recombinant PANK, PDH and ATF added thereto, and growth of the bacteria in a medium supplemented pantothenic acid, among other things.

San teaches two bacteria having exogenous ATF and differing activity of endogenous PANK. San does not teach bacteria having recombinant PANK or recombinant PDH and San does not teach supplementation with panthenoic acid. Yang does not teach a bacteria having recombinant PANK or recombinant PDH, but only a bacteria lacking *ackA-pta*. Voet does not

teach bacteria having **any** recombinant genes, only basic metabolic pathways. Vallari does not teach a bacteria having recombinant PANK or recombinant PDH, but only a bacteria having a mutant endogenous PANK that is resistant to feedback inhibition.

Therefore, even when combined, the art does not teach **every claimed element** and a *prima facie* case of obviousness is **not** made. Examiner has failed to cite art that teaches the missing elements of having added recombinant PANK and PDH. If the Examiner is relying on common or personal knowledge to supply the missing elements and motivation to combine, Examiner is again requested to put such material in proper evidentiary form for rebuttal.

The cited references do not teach supplementation with pantothenoic acid. As noted by Vadali, et al. (Metab. Eng. 6:133-9 (2004), at page 138), "It was found that the intracellular CoA/acetyl CoA could be increased **only** with the simultaneous overexpression of pantothenate kinase **and supplementation of pantothenic acid**. Since *E. coli* normally secretes out excess pantothenic acid, it might be logical to assume that the availability of precursor will not be rate limiting. On the contrary, the **supplementation of pantothenic acid is essential and necessary** for CoA/acetyl-CoA manipulation." (*emphasis added*) Thus at the time of filing, it was unexpected that bacteria would require supplementation with pantothenic acid and, without supplementation, increased CoA flux would not be achieved.

Further, even if the art did teach every recited element (and it does not), it is not obvious to make the combination suggested by the Examiner. As admitted by the Examiner at page 3 of the Office Action, "It is **not** clear to one having skills in the art, why the Applicants, aiming at the increase in CoA production in a transformed cell, transfect the cell with a [PDH] gene and with ATF2 gene. According to the state of the art at the time of filing, **both enzymes deplete the CoA pool**." Therefore, there is no motivation to make the suggested combination.

CONCLUSIONS

Applicants have amended the claims to recite "increasing Coenzyme A (CoA) flux." The claims as amended clearly describe the claimed invention. The cited art does not teach bacteria with recombinant genes encoding PanK, PDH **and** ATF, or "culturing said cell in a cell medium comprising **pantothenic acid**."

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. At the very least, claim 27 and its dependant claims appear to be free of the art and all 112 rejections adequately addressed. Therefore, Applicants request of at least these claims. Applicants respectfully request the Examiner contact them if there are any questions or issues that need to be addressed.

It is believed that no fees are required for this submission. Should Applicants be incorrect, please charge additional fees and credit any overpayment to Deposit Account No. 50-3420 (reference 31175413-005002 MDB)

Dated: March 29, 2007

Respectfully submitted,

BAKER & MCKENZIE LLP

By /Michael D Berger/

Michael D. Berger Ph.D., Patent Agent
Registration No.: 52,616
Pennzoil Place, South Tower
711 Louisiana, Suite 3400
Houston, Texas 77002-2746 USA
Tel: +1 713 427 5031
Fax: +1 713 427 5099